EVALUATION OF PERIODONTAL TISSUE RESPONSE TO NON-SURGICAL PERIODONTAL THERAPY IN PATIENTS WORK IN BASIC CHEMICAL INDUSTRIES AND FERTILIZERS FACTORY

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ABSTRACT

Background: Potential pollution of air, water and land is a consequence of fertilizers industrial productions that have biohazardous impact on animal, human health and ecosystem. Aim: To investigate the clinical response and the levels of IL-6 and Nitric Oxide derivatives (nitrogen oxide) (NO°) after non-surgical periodontal therapy in chronic periodontitis patients working in fertilizers factory. Material and Methods: 60 subjects were included in the study. Forty chronic periodontitis male patients equally divided into 2 groups. Group I, 20 patients working in the fertilizers and chemical industries factory and group II, 20 patients did not work or live near any fertilizer factories. Twenty healthy volunteers not working in fertilizer factories were act as a control group (Group III). The Plaque Index (Pl-I), Gingival index (GI), Probing pocket depth (PD), and the Clinical Loss of Attachment (CLA) (clinical parameters) were scored for all subjects at baseline and one month after non-surgical periodontal therapy. Gingival Crevicular Fluid (GCF) samples were collected from all subjects, used for analysis by RT-PCR to assess the levels of IL-6 and Nitric Oxide derivatives (nitrogen oxide) (NO°) before (baseline) and one month after periodontal therapy. Results: One month after periodontal therapy, the two groups responded well to the non-surgical treatment but non-significant difference was observed (P>0.05). GI and Pl-I scores were reduced (P<0.001) with reduction in PD and gain in clinical attachment (P<0.05) in both groups. At baseline, the GCF mRNA levels of IL-6 and NO° were higher in both studied groups when compared with the healthy group. But the levels were high in group I more than group II. The levels of mRNA for IL-6 and NO° were significantly reduced after periodontal therapy in group I and II. A significant correlation between the levels of mRNA for IL-6 and NO° derivatives with the clinical parameters mean scores at baseline and one month after periodontal therapy in both groups (P<0.05). Conclusion: Continuous exposure to fertilizers pollution did not affect the clinical response of non-surgical periodontal therapy but it affects the mRNA levels of free radicals NO° and the pro-inflammatory cytokine IL-6 that lead to more destruction and worse outcomes of periodontal tissues.

KEY WORDS: Periodontal therapy, fertilizers, IL-6, NO, periodontitis

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INTRODUCTION

Periodontitis is a chronic disease of periodontium results from bacterial infection of periodontal tissues that initiated by a bacterial biofilm on the tooth surface triggering immune response that leads to destruction of the alveolar bone supporting the teeth, this may takes many years to develop leading to tooth loss if not treated \(^{(1, 2)}\). Pro-inflammatory cytokines are released from stimulated host cells due to the effects of bacterial pathogens from subgingival dental plaque. These cytokines recruit neutrophils to the site of infection which secret proteolytic enzymes, such as elastase and O2 (molecular oxygen) by oxidative burst, thus it play a major role in the etiology of periodontal diseases \(^{(3)}\).

Free radicals constitute a group of diverse species that are highly reactive, include reactive oxygen species (ROS), reactive nitrogen species (RNS) and chlorine species that result from the cellular redox process and can oxidize various bio molecules essential to cell and tissue function by extracting electrons. Lipids, proteins and nucleic acid (DNA) are essential cellular biomolecules that can be altered by the effects of free radicals \(^{(4)}\).

It is a well-known fact that low concentration of free radicals are essential to many normal biologic processes. It help fibroblasts and epithelial cells to grow in culture, however, at higher concentrations, it may become injurious to the tissue \(^{(4, 5)}\). It is reported that free radicals have a role in pathogenesis of periodontal disease \(^{(6)}\).

The human body incorporates a plethora of complex antioxidant defense system that prevent the damaging effects of the harmful ROS/RNS as soon as they are formed. Therefore, there should be a balance between the antioxidants and the concentration of free radicals. ‘Oxidative stress’ is a state in which the levels of antioxidants are diminished while the formation of free radicals are increased that is if the stress is massive and prolonged can result in serious cell damage and therefore development of many chronic and degenerative diseases including periodontal diseases \(^{(5-8)}\).

Nitric oxide (NO) is a gaseous lipophilic, free radical of short life span implicated in neurotransmission, vasodilatation and immune regulation; however, it is relatively unstable and rapidly auto-oxidized in the presence of oxygen to produce nitrogen oxides \(^{(8,9)}\). NO is produced from L-arginine through the action of isoenzymes, generated constitutively in small amounts by two distinct isoforms of NOS, neuronal (nNOS) and endothelial NOS (eNOS) whereas, it is induced in area of inflammation by another inducible NOS isoform (iNOS) \(^{(9)}\). It is reported that oxidative stress impaired NO bioavailability and vascular dysfunction and the defective production of NO has been reported in pathogenesis of many disease processes including periodontitis \(^{(6,9,10)}\). Interleukin 6 (IL-6) is a pro-inflammatory cytokine regulating immune and inflammatory response \(^{(11)}\). IL-6 induce indirect osteoclastogenesis by stimulation of osteoblast and synovial cells to increase the secretion of RANKL \(^{(9)}\). Therefore, IL-6 is associated with bone destruction in advanced periodontal disease \(^{(12, 13)}\). Furthermore, IL-6 increases secretion of matrix proteinase enzyme (MMPs) in periodontitis \(^{(9)}\).

The ROS peroxynitrate and hydroxyl radical target cellular DNA leading to DNA abnormalities such as strand breaks, mutations, deletions, insertions, nicking and sequence amplifications which may cause damage to different cellular structures and disease process \(^{(14)}\).

Nowadays, human has huge optimistic benefits and a progressive improvement of life-welfare form industrialization and manufacturing; however, the hazardous by-products (chemical contamination) have deleterious effects to the environment and human health. Fertilization industry produce fertilizers that are widely used in agricultural activities to obtain better quality of agriculture products. However, the production of fertilizers affect negatively on the environment leading to
potential pollution of air, water and land. In each part of the production chain processes hazards are expected and should be avoided by occupational safety measures for all people in close vicinity to these products. As reported previously, multifactorial diseases affect about 15-35% of adult population in industrialized countries. Cells of human body exposed to oxidants originating from a large variety of sources either endogenous or exogenous (environmental). The endogenous sources are the bi-products of physiological cell activities, which result in formation of ROS. On the other hand, exogenous sources include environmental factors as extreme temperature changes, UV light, cigarette smoking, drugs, gases, radiations, infections, all are oxidants.

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Numerous amounts of intracellular free radicals (ROS, RNS) are produced by metabolic process of various atmospheric environmental pollutants, organic compounds and metals causing oxidative damage to vital biomolecules. This damage result in a pro-inflammatory condition with cytotoxic effects that mediated by the redox-sensitive mitogen-activated protein kinase (MAPK) and NF-κB cascades leading to expression of several pro-inflammatory cytokines and adhesion molecules, released in inflammation. Hence, occupational exposure to atmospheric pollutants from fertilizer industry may increase risk of chronic periodontitis.

The aim of this study is to investigate the response of periodontal tissue and the mRNA expression levels of IL-6 and Nitric Oxide derivatives (nitrogen oxide) (NO°) after a non-surgical periodontal therapy in chronic periodontitis patients work in fertilizer factories.

SUBJECTS AND METHODS

Subjects

This study was conducted on 40 adult males suffering from moderate to severe chronic periodontitis. These subjects were divided into two groups according to the international workshop for classification of periodontal diseases and conditions in 1999. Group 1, 20 patients suffering from chronic periodontitis and working in fertilizers and basic chemical industries factory in Helwan district, Cairo, Egypt with minimum of 5 years working period. Group II, 20 patients suffering from chronic periodontitis and did not work or even live near any fertilizer factories. Twenty healthy volunteers’ subjects not working in fertilizer factories were included in the study as a control group (Group III) with age range 30 – 45 years.

Inclusion criteria

Presence of at least 10 periodontal pockets with minimum depth 5 mm for chronic periodontitis, non-smokers, no history of antibiotic therapy during 6 months earlier, systemically free and age ranges from 30-55 years.

Informed consent

Full signed consents were obtained from all patients before beginning of the study after they informed about the nature and objectives of the study. The study followed the rules of Good Clinical Practice Guidelines set by the International Conference, and the Declaration of Helsinki. Ethical Committee of College of Dentistry, Minia University in Egypt reviewed and approved the study protocol.

Clinical parameters

The clinical parameters recorded for each patient were the followings: Plaque Index (PI-I), Gingival index (GI), Pocket depth (PD), Clinical Loss of Attachment (CLA). Radiographic examination (periapical radiographs) were performed using long cone parallel technique to determine the degree of bone loss.

GCF samples collection and analysis

For every patient and control subject Gingival Crevicular Fluid (GCF) samples were collected
from 3 periodontal sites in three different quadrants with attachment loss 5 mm or more. Isolation of the sampling area was done by cotton rolls then cleaned supragingivally. A sterile paper points were inserted into the full depth of gingival crevices or pockets. After 30 seconds, the paper points removed from the pockets then transferred to 100 µL of distilled water (DW) and mixed vigorously. Samples without the paper points centrifuged, and washed twice with DW. The supernatant pellet was re-suspended in 0.4 mL of DW, stored at –70°C to be used for PCR analysis.

**Periodontal therapy**

Scaling and root planing for every subject were performed using ultrasonic scaler and periodontal curettes in three sessions within 10 days period. A maintenance program was described to all patients and they were instructed to follow strictly. This program include regular using a soft toothbrush and toothpaste (Formula system) two times daily and the use of interdental toothpicks. Chlorhexidine mouthwash (Hexitol), 500 mg Amoxicillin and 250 mg metronidazole (Flagyl) were prescribed and patients were instructed to use it 3 times / day for 7 days. The patients of group I and group II were followed up at 2 weeks intervals. The sampling procedures were repeated for every patient one month after periodontal therapy.

**RT-PCR analysis**

**RNA extraction**

RNA was extracted using denaturing solution (solution D) formed of the following reagents: - 250g Guanidinium thiocyanate dissolved in 293 ml Water. 17.6 ml of 0.75 M sodium citrate (pH7). 26.4 ml 10% sarcosyl. 0.36 ml of 2- mercaptoethanol. Phenol water saturated at 4°C. The sample was defreezed to room temperature and suspended in 1 ml of solution D in disposable Eppendorf tube and the following reagents was added and mixed by inversion after addition of each reagents:- 0.1 ml of 2 M sodium acetate (pH4), 1 ml of water saturated phenol, 0.2 ml of chloroform- isoamyl alcohol mixture (49: 1). The final suspension was shaken vigorously for 10 Sec. and cooled on ice for 15 minutes, then were centrifuged at 10000 g (14000 R P M) for 20 min at 4°C (in MIKRO 22 R centrifuge). After centrifugation, three phases appeared in the tube; an aqueous phase (containing RNA), phenol phase (containing phenol) and the interphase (containing DNA, proteins and other solid ingredients).

The aqueous phase was transferred to a new fresh tube using micropipette, double volume of isopropanol was added, and the mix kept for 1hr at –20°C to precipitate RNA. Sedimentation of RNA was performed by centrifugation of the cooled aqueous material for 20 minutes at 4°C. The sedimented RNA pellet was again dissolved in 0.3 ml of solution D and transferred into a new 1.5 Eppendorf tube and centrifuged for 10 minutes at 4°C to precipitate RNA pellet again. The formed RNA pellet was suspended in 75% ethanol and vacuum dried. The sedimented RNA pellet was dissolved in 500μl H2O to be used for PCR assay.

**PCR Assay**

10x - PCR reaction buffer (Tris-HCl 100mM - KCl 500mM - MgCl2 15mM) The pH of the buffer was adjusted to 8.3 (at 25°C) , Gel loading buffer (0.2 % Bromophenol blue - 0.2 % Xylene Cyanol - 50% Glycerol), deoxynucleotide Tri- Phosphate mix (dTTP) (10mM from dATP, dCTP, dGTP and dTTP) , DNA polymerase (AmpliTaq).

**Primer Sequence:**

The specific primers were prepared Clinilab (Clinilab, Import, Export, Agencies. Maadi, Cairo, Egypt). The sequence of PCR primers for IL-6 were 5’CTCAGTTTGCTGCGAAGGTTCACTT3’ and 5’GCTGGGGTGTGCTGGGTTTG’ with 186 bp PCR product size. For Nitrogen oxide (NO• derivative), the sequence of PCR primers...
were 5’ATGACATTTGGGACATCGG3’ and 5’AATGGGGTTAGGTTCGTGG5’ with 327 bp PCR product size. 50 µl was used for PCR, containing 10 µl of solution DNA Pre-mix buffer (50mM KCl, 10mM Tris-HCl pH 8.4, 0.1% Triton X-100, 1.5mM MgCl2, deoxynucleoside triphosphates, Taq DNA polymerase) and 20pmol/reaction of the PCR primers.

The temperature setting of PCR reaction for IL-6 were; denaturation at 94°C for 3 min, 35 cycles of annealing at 94°C for 30 sec, extension at 47°C for 35 sec and 80°C for 30 sec. The final extension period at 72°C for 5 min. The temperature settings of the PCR reaction for Nitric oxide (NO•) were; denaturation at 94°C for 3 min, 35 cycles of annealing at 60°C for 15 sec, extension at 70°C for 20 sec and 88.5°C for 30 sec. The final extension period at 72°C for 5 min. Amplification was performed in a PTC-100-60 thermocycler (MJ Research, Waltman, MA, USA). The PCR products were digested with 5 U of Taq I DNA polymerase at 65°C for 4 h.

0.5 µL of DNA primers at the same base position were included as a positive control used in a separate microcentrifuge tube. PCR performed without GCF sample (distilled water only) was used as a negative control.

The detection of the PCR product was performed by using agarose gel electrophoresis. 20 µL of sample (PCR product) was loaded in each Well for electrophoresis. The agarose gel containing 1.5% agarose and 0.5µg/ml ethidium bromide with 0.5X Tris- acetate EDTA buffer. Electrophoresis was conducted at 4Volt/cm³ of the gel (60-80Volt for the total gel piece) for 1-1.5hrs. running within the gel a molecular DNA size marker for matching the base-pairs (bp) position of positive PCR assays. The DNA was photographed under 312 nanometer (nm) Ultraviolet light transilluminator.

After detection of the PCR product 5µl of the positive samples was used for the quantitative analysis of IL-6 and Nitrogen oxide (NO•) in the form of picogram/µl. Quantitation was carried out by the use of the Bright – Glo™ Luciferase assay system developed by Promega® in TD- 20/20 – Luminometer. Luciferase is an enzyme used for assessment of the quantity of marker (in culture and serum), amplified DNA and RNA by giving a luminescence signal that can be counted by the use of Luminometer. The temperature of the Bright – Glo reagent was held to the room temperature which was near the optimum temperature of luciferase. Then 100µl of Bright- Glo Luciferase was added to 5µl of the sample to be used in quantitation and 5µl of luciferase assay buffer was added to the tube and left at room temperature for 2 min., then measured in the luminometer.

**Statistical Analysis:**
All data were tabulated and used for statistical analysis using Statistical Package for Software System Program (SPSS version 15.0 Inc., Chicago).

The clinical parameters (GI, PI-I, PD and CLA) were scored from every patient before and after periodontal therapy. The mean values of clinical parameters were computed and evaluated using paired t -test and levels at P<0.05 were considered significant. The frequency of mRNA expression for IL-6 and Nitric oxide (NO•) per group and difference from baseline to one month after periodontal treatment was assessed by Chi-square test and levels at p<0.05 were considered significant. Differences in the mean levels of IL-6 and Nitrogen oxide (NO•) primers PCR mRNA expression from baseline to one month after periodontal therapy was evaluated by paired t-test. Spearman rank correlation test was used to study the correlation between the mean scores of clinical parameters and the mean levels of IL-6 and Nitrogen oxide (NO•) at baseline and one month after periodontal therapy. Levels at P<0.05 were considered significant.
RESULTS

Clinical parameters

The healthy control group comprised 20 male subjects, with age ranged from 30 - 45y and the mean age was 31.11 ± 4.28y. The mean GI and Pl-I scores was below 1 and mean crevice depth was 1.76 ± 0.47 with no loss of attachment (Table 1).

<table>
<thead>
<tr>
<th>Age</th>
<th>GI</th>
<th>Pl-I</th>
<th>Crevice depth</th>
<th>CLA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ±SD</td>
<td>31.11 ±4.28</td>
<td>0.62 ±0.23</td>
<td>0.67 ±0.23</td>
<td>1.76 ±0.47</td>
</tr>
</tbody>
</table>

**Group I** (chronic periodontitis patients working in the fertilizers and basic chemical industries factory) comprised 20 male patients, with age range 30 – 53y and the mean age was 41.5 ± 4.37y. At baseline the mean GI scores was 2.12 ± 0.53 and the mean PI-I scores was 2.53 ± 0.44. The mean pocket depth was 4.32 ± 0.75 mm and the mean loss of clinical attachment was 5.44 ± 1.63 mm. After periodontal therapy there was obvious reduction in PI-I and GI scores (**P<0.001**) and statistically significant decrease in PD and improve in clinical attachment (**P<0.05**) (Tables 2, Fig 2).

On comparing the response to periodontal therapy a non-significant difference between the studied 2 patients groups was observed (**P>0.05**), both groups responded well to the non-surgical treatment (Fig 3).

**Group II** (chronic periodontitis patients not working in the fertilizers and basic chemical industries factory) comprised 20 male patients with age range 30 - 48y and mean age 46.75 ± 4.62y. At baseline the mean GI scores was 1.91 ± 0.29 and mean crevice depth was 1.89 ± 0.49, the mean PD was 4.81±0.65 with mean loss of attachment 5.74 ± 0.52 mm. One month after periodontal therapy both GI and PI-I scores were reduced (**P<0.001**) with reduction in PD and gain in clinical attachment (**P<0.05**) (Table 1).

**TABLE (2)** The clinical parameters mean scores in group I before and after periodontal therapy.

<table>
<thead>
<tr>
<th>Before therapy (X± SD)</th>
<th>After therapy (X± SD)</th>
<th>Mean difference (X± SD)</th>
<th>t- Value</th>
<th>P- Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GI</td>
<td>1.91 ± 0.29</td>
<td>0.92 ± 0.22</td>
<td>0.99 ± 0.16</td>
<td>12.7</td>
</tr>
<tr>
<td>Pl-I</td>
<td>1.89 ± 0.49</td>
<td>0.96 ± 0.43</td>
<td>0.93 ± 0.16</td>
<td>11.60</td>
</tr>
<tr>
<td>PD</td>
<td>4.81 ± 0.65</td>
<td>3.37 ± 0.54</td>
<td>1.44 ± 0.24</td>
<td>6.12</td>
</tr>
<tr>
<td>CLA</td>
<td>5.74 ± 0.52</td>
<td>4.45 ± 0.72</td>
<td>1.29 ± 0.39</td>
<td>4.54</td>
</tr>
</tbody>
</table>

**TABLE (3)** The clinical parameters mean scores in group II before and after periodontal therapy.

<table>
<thead>
<tr>
<th>Before therapy (X± SD)</th>
<th>After therapy (X± SD)</th>
<th>Mean difference (X± SD)</th>
<th>t- Value</th>
<th>P- Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GI</td>
<td>2.12 ± 0.53</td>
<td>1.01 ± 0.22</td>
<td>1.11 ± 0.31</td>
<td>5.8</td>
</tr>
<tr>
<td>Pl-I</td>
<td>2.53 ± 0.44</td>
<td>0.98 ± 0.35</td>
<td>1.55 ± 0.09</td>
<td>7.65</td>
</tr>
<tr>
<td>PD</td>
<td>4.32 ± 0.75</td>
<td>3.12 ± 0.62</td>
<td>1.20 ± 0.13</td>
<td>6.5</td>
</tr>
<tr>
<td>CLA</td>
<td>5.44 ± 1.06</td>
<td>4.31 ± 0.94</td>
<td>1.13±0.12</td>
<td>5.76</td>
</tr>
</tbody>
</table>
EvAluaTion of periOdontaL tissue reSpoNSe to non-surGical periOdontaL therapy

Expression of IL-6 and NO derivative

The levels of IL-6 and NO$^*$ derivatives were higher in both studied groups when compared with the healthy group. The levels of both molecules were also higher in group I (workers in fertilizers and basic chemical industries factory with chronic periodontitis) than in group II (non-workers in fertilizers and basic chemical industries factory). There was significant difference when the levels of both molecule compared before and after non-surgical periodontal therapy in both studied groups. It was $P < 0.05$ in group I and $P < 0.001$ in group II (Table 4, Figs 4,5).

TABLE (4) Levels of IL-6 and NO mRNA expression in both studied patient groups and healthy control group.

| Study Groups | IL-6 pg/µL | | NO pg/µL | |
|--------------|------------|------------|------------|
|              | Baseline   | After Therapy | P       | Baseline | After Therapy | P       |
| GI           | 214.65±35.28 | 135.18±78.23  | <0.05 | 164.65±23.67 | 124.15±23.67  | >0.05 |
| GII          | 136.15±56.2  | 64.52±42.3  | <0.001 | 98.77±12.54  | 47.77±52.4  | <0.001 |
| P            | <0.05       | <0.001      |        | <0.001      | <0.001      | ---    |
| Healthy Group| 87.26±23.67  | -          | -       | 39.44±5.7   | -          | -      |
| P            | <0.001      | -          | -       | <0.001      | -          | -      |
Correlation of IL-6 and NO expression with the clinical parameters

The correlation of the levels of mRNA for IL-6 and Nitrogen Oxide with the clinical parameters in group I (workers in fertilizers and basic chemical industries factory with chronic periodontitis) and group II (non-workers in fertilizers and basic chemical industries factory) before and after periodontal therapy are summarized in table (5,6). The levels of mRNA for IL-6 and NO were significantly correlated with the clinical parameters mean scores at before and one month after periodontal therapy in group I ($P<0.05$). Considering group II a significant direct positive correlation between the levels of mRNA for IL-6 and NO with all the clinical parameters before periodontal therapy ($P<0.05$). Such significant correlation persists one month after periodontal therapy ($P<0.05$) with the levels of IL-6 but the levels of NO showed a direct positive correlation after periodontal therapy with all the clinical parameters ($P<0.05$).

TABLE (5) Correlation Coefficient ($r$) between the mean levels of IL-6 mRNA and NO mRNA with the mean scores of clinical parameters before and after periodontal treatment in group I.

<table>
<thead>
<tr>
<th>Date</th>
<th>Mean levels of IL-6 and NO mRNA in Pg/µL</th>
<th>GI X ±SD</th>
<th>PI-I X±SD</th>
<th>PD X ± SD</th>
<th>CLA X± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before periodontal Therapy</td>
<td>IL-6 214.65±35.28</td>
<td>0.465</td>
<td>0.487</td>
<td>0.611</td>
<td>0.615</td>
</tr>
<tr>
<td></td>
<td>NO 164.65±23.56</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>After periodontal Therapy</td>
<td>IL-6 135.18±78.23</td>
<td>0.476</td>
<td>0.543</td>
<td>0.572</td>
<td>0.491</td>
</tr>
<tr>
<td></td>
<td>NO 124.15±23.3</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>
The present study investigated the clinical responses and the mRNA levels of pro-inflammatory cytokine IL-6 and the derivatives of the free radical NO° (nitrogen oxide) in gingival crevicular fluid of patients suffering from chronic periodontitis working in fertilizer factory after non-surgical periodontal therapy in comparison with chronic periodontitis patients’ not working in fertilizers factory. The oral cavity are exposed to many factors including environmental, occupational, dietary, pathological and oral hygiene condition that affects the health and conditions of the oral and dental tissues.

Workers in fertilizer and chemical industries are potential of occupational exposure to hazardous materials primarily through the inhalation of fertilizer dusts and dermal routes that affect negatively on their general health (25). When the medical healthcare facilities especially oral healthcare facilities are deficient, employees in fertilizer and chemical industry factory are prone to bad general and oral health conditions.

The treatment protocol was scaling and root planing to eradicate infected cementum and to minimize the pathogenic bacteria in periodontal pocket combined with systemic antibiotic therapy consists of amoxicillin, which is very effective against most periodontal pathogens (26,27) and metronidazole that combat the anaerobic microorganisms. Systemic antibiotics were used to rapidly reduce targeted microbes, accelerate the healing of inflamed periodontal tissues and regain host compatible microflora.

There were significant improvement of clinical parameters in treated patients after one month of non-surgical periodontal therapy and the patients groups responded well to the treatment protocol. The clinical parameters decreased significantly when compared with the baseline parameters. These findings are in agreement with the previous results (28, 29).

The expression levels of mRNA of Nitrogen Oxide and IL-6 in GCF were higher in patients of two studied groups when compared with healthy group. However, the levels were more higher in workers...
in fertilizer factory than in non-workers. After non-
surgical periodontal therapy the levels of NO and IL-6 were decreased significantly in both groups. The levels of NO and IL-6 were significantly higher in group I when compared with group II.

Our findings suggested that the increased level of (NO˚) in patients working in fertilizer factory might be partly due to the increased level of pro-inflammatory cytokines (iNOS) and subsequent periodontal tissue destruction, and partly due the occupational exposure to fertilizers dusts and fumes during production process. This leads to increase of free radicals including (NO˚) that promote the oxidative stress, which have destructive impact on the periodontal tissues. It is reported that oxidative stress impaired NO bioavailability, vascular dysfunction and altered NO production predispose to periodontal tissue destruction (9,10). In previous study, a trend of increased IL-6 expression observed in GCF of periodontal tissues in patients suffering from periodontitis and more significantly increased in diabetic patients with periodontitis (30).

Previous studies have reported a strong association between chronic periodontitis and the many pro-inflammatory cytokines as regards to protein expression and mRNA levels in GCF, gingival tissue and serum. These cytokines are tumor necrosis factor-alpha (TNF-α), inducible nitric oxide synthase (iNOS), Nitric Oxide (NO˚), IL-6, and IL-1β. These studies found that the increased production of IL-6 and NO in periodontal, and gingival tissues of chronic periodontitis patients have also enhanced expression of protein and an increased level of iNOS mRNA (9, 13, 30,31). The endogenous sources of IL-6 and iNOS are mainly from the immune cells, endothelial cells and fibroblasts (32).

In the present study a positive correlations was found between the levels of both IL-6 and (NO˚) and the scores of clinical parameters before and after one month of non-surgical periodontal therapy in both groups. Therefore, indicating that the non-
surgical periodontal therapy is effective and efficient in reducing higher scores of clinical parameters as well as the levels of pro-inflammatory mediators IL-6 and (NO˚).

CONCLUSION

Prolonged exposure to dusts and fumes of fertilizers is the main occupational health problem in fertilizer manufacture, which may be a risk factor for periodontitis and periodontal destruction. It does not affect the clinical outcomes of nonsurgical periodontal therapy.

RECOMMENDATION

Further studies are required to evaluate the free radicals and antioxidants levels in patients working in fertilizer factories and to clarify the molecular mechanisms by which the fertilizer dusts affects the levels of pro-inflammatory mediators in GCF.

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